Published in final edited form as:

Adv Exp Med Biol. 2014 ; 806: 283–299. doi:10.1007/978-3-319-06068-2_12.

Mass Spectrometry-Based Tissue Imaging of Small Molecules

Carly N. Ferguson¹, Joseph W.M. Fowler¹, Jonathan F. Waxer¹, Richard A. Gatti^{2,3}, and Joseph A. Loo^{1,4}

¹Department of Chemistry and Biochemistry, University of California-Los Angeles, Los Angeles, CA, USA 90095

²Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, CA, USA 90095

³Department of Human Genetics, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, CA, USA 90095

⁴Department of Biological Chemistry, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, CA, USA 90095

Abstract

Mass spectrometry imaging (MSI) of tissue samples is a promising analytical tool that has quickly become associated with biomedical and pharmacokinetic studies. It eliminates several labor-intensive protocols associated with more classical imaging techniques, and provides accurate, histological data at a rapid pace. Because mass spectrometry is used as the readout, MSI can be applied to almost any molecule, especially those that are biologically relevant. Many examples of its utility in the study of peptides and proteins have been reported; here we discuss its value in the mass range of small molecules. We explore its success and potential in the analysis of lipids, medicinals, and metal-based compounds by featuring representative studies from mass spectrometry imaging laboratories around the globe.

Introduction

Mass spectrometry-based imaging of tissue samples and surfaces is a relatively new technology with a significant function in medicinal development (McDonnell and Heeren, 2007b). MSI was developed in the laboratory of Dr. Richard Caprioli, and has continued to garner interest in labs around the world as its applications expand (McDonnell and Heeren, 2007b, Reyzer and Caprioli, 2011). The methodology essentially provides a chemical map of a surface by incorporating mass spectrometry instrumentation and assigning spatially relevant coordinates (McDonnell and Heeren, 2007b). The specificity of analyte detection is provided by the mass spectrometry readout.

MSI is most often coupled with matrix-assisted laser desorption/ionization (MALDI), although we will briefly discuss an alternative ionization method. MALDI utilizes a matrix compound that is mixed and crystallized with the analyte of interest followed by desorption/ionization with a laser beam. MALDI matrices absorb efficiently at the wavelength of the specified laser. In MSI, the fundamentals of MALDI are utilized in a spatially relevant manner. A thin surface (for our purposes, a thin tissue section) is evenly coated in a MALDI

matrix, and this surface is subsequently analyzed by rastering across it with a MALDI laser irradiation. The spatial resolution is controlled by the operator and is limited by the laser spot size (e.g., typically 100 μm or less), and thousands of spectra are collected at specific X-Y coordinates covering the surface of interest. After collection, all spectra are compiled into one, average spectrum, wherein masses of interest can be selectively highlighted and their spatial distribution depicted in a virtual image (McDonnell and Heeren, 2007b). This workflow is shown in Figure 1. Tissue samples (whole organs) are typically sliced on a cryotome at 10 – 20 μm thickness and thaw-mounted onto glass slides coated with conductive material. Matrix is applied via any one of a number of automated mechanisms, typically consisting of a nebulizer that evenly coats the entire slide with a thin layer of matrix crystals (McDonnell and Heeren, 2007a, Reyzer and Caprioli, 2011, McDonnell and Heeren, 2007b).

There are several benefits of implementing mass spectrometry imaging. Traditional protein and peptide imaging methods, such as immunohistochemistry (IHC) staining, require the use of a specific antibody (Coons et al., 1942). Additionally, these staining methods only allow for a small number of molecules of interest to be analyzed on any given tissue section (Andersson et al., 2008, Sanchez-Carbajo, 2006). MSI does not require antibodies, and the use of a mass spectrometer's analyzer and detector also allow for the visualization of thousands of molecules of interest simultaneously as all ionized molecules are non-specifically detected. Similar benefits are true of MSI experiments for detection of small molecules. The mass accuracy of the MS analyzer, e.g., time-of-flight (TOF) analyzers most often applied for MSI, often allows for the identification of the molecule of interest, and advanced MS methods, such as tandem mass spectrometry (MS/MS), yield additional molecular information to derive identification.

A recent study by the Caprioli group demonstrates the functionality of MSI in a pathological sense. This work probed proteomic differences between two dermatological conditions: Spitz nevus (SN) and Spitzoid malignant melanoma (SMM). These two conditions are virtually indistinguishable by traditional pathology methods; however, an MSI study was able to identify a key proteomic difference that distinguishes the benign SN from the malignant SMM with 97% accuracy. This study showcases the ability of MSI to complement existing histopathology methods for more confident diagnoses (Lazova et al., 2012).

In addition to interest in proteins and peptides, small molecules of all types are compounds of interest in many clinically relevant studies. Herein we discuss the advances of mass spectrometry imaging in three classes of small molecules: lipids, drug compounds, and nanoparticles. Combined, these studies broadly indicate the bright future MSI holds in the biomedical field.

Lipids

Lipids have considerable diversity in chemical structure and biological function, and comprise the external lipid bilayer as well as subcellular organelles, including the mitochondria and surrounding nuclear membrane (Fahy et al., 2005). Due to this diversity in

structure and localization, techniques that pinpoint specific lipids within tissues, while not delocalizing the compound of interest, are of great interest. The techniques generally used to identify lipids, however, involve the extraction of the lipids prior to analysis, which destroys relevant information regarding its overall spatial distribution (Murphy et al., 2009). These traditional procedures for lipid analysis utilize destructive assays, which are subsequently coupled with mass spectrometry and/or liquid chromatography (Bulley et al., 1984, Conte et al., 1997, García-Ayuso et al., 2000, Taylor et al., 1993, Wenk, 2005). Fluorescence imaging could theoretically overcome these challenges, as fluorescent tagging procedures preserve location, reveal the exact position of lipids, and allow researchers to observe the rapid and dynamic changes in the location and structure of lipids (Schultz et al., 2010). However, most fluorescent tags are as large as the lipid molecules themselves, which would likely cause changes to the overall location and metabolism of the lipid. Therefore, in order to successfully study lipids within a biological system, analytical techniques must be able to overcome two major challenges: (i) preservation of the structural and locational information of individual lipids, and (ii) provide a high level of molecular specificity to differentiate the diversity in lipid structure (Ellis et al., 2013). Modern mass spectrometry, due to its excellent sensitivity and molecular specificity, is arguably a method of choice for lipid analysis. The mass spectrometric analysis of lipids is traditionally achieved through lipid extraction from a sample and subsequent analysis by electrospray ionization (ESI), with or without prior chromatographic separation (Ellis et al., 2013). Although this method provides detailed structural information, it is not useful for measuring the spatial distribution of specific lipids. Therefore, new mass spectrometry approaches have been developed that provide direct surface analysis capabilities.

MALDI-MSI of Lipids

Recently, advances in MALDI-MSI have allowed for in-depth qualitative, quantitative, and spatial analyses of several types of lipid species. Although the preparation and specific parameters needed to map lipids varies between specific species, MALDI-MSI has been shown to be a more efficient and convenient technique to detect lipids compared to traditional methods. Techniques, such as fluorescence confocal microscopy and fluorescent tagging of molecules are poor in lipid characterization because of the relatively small size of lipids as well as their ubiquity throughout many cell types (Zemski Berry et al., 2011). The abundance of lipids in various cell structures, such as internal and external membranes, is beneficial to MALDI IMS because it allows for the direct visualization of these various compounds on a tissue section. Furthermore, the sensitivity of MS for molecular weight ranges under 1000 Da is especially high (Börner et al., 2007).

MALDI generates two-dimensional molecular maps by ionizing molecules on a tissue sample through direct exposure to a laser. This is ideal for analysis of lipid compounds because of their inherent ability to ionize and produce positive or negative ions. Lipid molecules are amphipathic molecules comprised of either phosphate anions or nitrogen-centered cations that readily ionize during MALDI (Chaurand et al., 2006, Fahy et al., 2005).

Several techniques exist to prepare various types of lipid species in order to obtain good quality mass spectra. Generally, washing the mounted tissue slides with aqueous, volatile salt solutions simplifies the spectra to lipid compounds, such as phosphatidylcholine, that generate an abundance of positive ions through MALDI (Wang et al., 2011). This will remove interfering salts and generate well-resolved mass spectra by taking out interfering chemical noise. Lipid species that form a high abundance of negative ions during the MALDI process, such as phosphatidylethanolamine, naturally produce simpler spectra because they do not form alkali metal attachment ions (Zemski Berry et al., 2011).

A final advantage of MALDI-MSI of lipids is that it can be used in combination with other techniques. For example, MALDI can be used after thin layer chromatography (TLC) allowing for precise isolation of lipid compounds directly on the TLC plate, versus a more labor-intensive extraction process (Zemski Berry et al., 2011). MALDI-MSI has also been used in conjunction with structural information obtained from ESI experiments, immunoblotting, and histological information obtained from staining to provide a multimodal model of lipid localization in the brain (Colsch et al., 2011). MALDI-MSI analysis of lipids has also allowed advancements in forensic investigation as well as diagnosis of diseases such as breast cancer and traumatic brain injury (Woods et al., 2013, Zemski Berry et al., 2011). MALDI-MSI has proven invaluable in the growth of lipid research and continues to spread into other avenues of biochemistry and analytical chemistry.

DESI of Lipid Compounds

MALDI-MSI is often used for peptide and protein analysis, and has been used for lipid analysis as well (Watrous et al., 2011). MALDI-MSI utilizes a preparative step that evenly covers the tissue with an organic matrix, assisting with ionization of the sample (Ferguson et al., 2011, Schwamborn and Caprioli, 2010). The type of matrix used, however, greatly influences the efficiency of ionization for different classes of molecules, and experimental conditions used for lipid imaging are different from those used for proteins in terms of matrix used, mass-to-charge (m/z) range, and the acquisition mode when using TOF analyzers (Eberlin et al., 2011a, Ferguson et al., 2011, Fuchs et al., 2010, McCombie and Knochenmuss, 2004, Schwamborn and Caprioli, 2010).

Desorption electrospray ionization (DESI) is an ambient desorption/ionization technique that is based on the direct examination of unprepared, unmodified samples in the open environment, and is commonly used for drug, metabolite, and lipid imaging. Recently, the development of dimethylformamide (DMF)-based solvent combinations has minimized the destructive nature of the technique, and has enabled DESI-MSI to be performed with preservation of morphological features (Watrous et al., 2011, Eberlin et al., 2011b). By preserving morphological features, histological and immunohistochemical analysis can be performed after MSI (Eberlin et al., 2011a). The ability to preserve tissue morphology for histological examination, while assessing the lipid and protein profiles on the same section of tissue, provides an opportunity to correlate findings (Eberlin et al., 2011b). Eberlin and Liu *et al* demonstrated that a single tissue section initially used for DESI-MSI of lipids can

then be used for protein MSI, and subsequently hemotoxylin and eosin (H&E) staining to acquire morphological information (Eberlin et al., 2011b).

By combining the techniques of DESI-MSI, MALDI-MSI, and H&E staining, Eberlin, et al. (2011b) unambiguously matched the morphological and chemical features of mouse brain and human brain cancer tissue samples. They found that, prior to DESI-MSI of lipids, using either acetonitrile (ACN):DMF (1:1) or ethanol:DMF (1:1) solvent systems did not disturb the native protein localization, as ion images of control tissue sections showed similar protein spatial distributions, as well as similar co-localization of lipids and proteins (Eberlin et al., 2011b). Furthermore, results were able to be obtained from a sample of human glioma grade III, having first undergone DESI-MSI, followed by MALDI-MSI, and finally optical imaging after H&E staining (Eberlin et al., 2011b). In addition, their analysis of a control tissue section, which had not been subjected to DESI-MSI, revealed similar spatial distributions of their select proteins of interest (Eberlin et al., 2011b). This new approach combines the unique strengths of DESI and MALDI for lipid and protein MSI, and allows the unambiguous matching of morphological and chemical features. Eberlin, et al. (2011b) conclude that the combined methods of DESI, MALDI, and H&E on the same tissue section enables a more complete evaluation; and, is expected to not only enhance diagnostic capabilities, but allow insights into the pathophysiology of disease.

These capabilities were further demonstrated by the same group in a study that employed ambient ionization-based MSI for characterization of tumor borders. Several brain tumors were analyzed via DESI-MSI to develop lipid-based classifiers to distinguish between tumor and healthy tissue. This technique provides fast classification, and remains promising for intraoperative use to differentiate visibly indistinguishable tumor borders (Eberlin et al., 2013).

MSI with FT-ICR MS for Lipid Analysis

MALDI-MSI with TOF analyzers does not provide the ultra-high resolution and mass accuracy necessary for direct identification of low molecular weight compounds. However, MALDI can be coupled with Fourier transform ion cyclotron resonance (FT-ICR) MS in order to obtain high mass accuracy and identify compounds of interest with a high degree of confidence in tissue (Marshall et al., 1998).

Vidova, et al. (2010) characterized the major lipid components of the ocular lens using MALDI-TOF-MSI. They found that the major lipid components were mainly long chain phosphatidylcholines (PC) and sphingomyelins (SM) (Rujoi et al., 2004). However, MALDI-TOF-MSI did not provide the proper degree of resolution and mass accuracy to determine the exact masses of the lipid components in order to produce accurate spatial distribution results of compounds close in molecular weight. Therefore, Vidova, et al. (2010) used MALDI-FT-ICR-MSI in order to determine the masses of the specific PC and SM species within the ocular lens. Sections of porcine eyes were prepared and analyzed with MALDI-FT-ICR-MSI in positive ion mode. An example of the usefulness of FT-ICR MS in this specific study was in the analysis of a lipid compound with a mass of 787 Da. The study found that the lipid aggregated in its protonated form at m/z 787.6685 and also found an unknown molecule at m/z 787.6042. Ultra-high resolution MS allowed for the

discrimination between these two peaks, whereas a lower resolution MS instrument would not be able to resolve the two, and would disrupt quantitative, qualitative, and spatial distribution analyses. By utilizing the high mass accuracy of FTICR MS, Vidova, et al. (2010) was able to find spatial and concentration-dependent distributions of various species of lipids within the ocular lens.

Lipids in Traumatic Brain Injury

MALDI-MSI is also an important tool that can be used to develop molecular biomarkers of disease. Recently, Woods, et al. (2013) used MALDI-MSI to characterize and quantify the spatial distribution of ganglioside species in mouse brains after being subjected to low level explosive detonations. This study set out to find a biochemical connection between exposure to explosive blasts and traumatic brain injury (TBI). Gangliosides account for 6% of total brain mass and are included in several biochemical and metabolic pathways (Holthuis et al., 2001). Ceramides are the scaffold molecules that form gangliosides, and the effects of proximity blasts from explosions may cause a disruption in the various pathways of these two lipids (Buccoliero and Futerman, 2003). Woods, et al. (2013) studied the changes in the amount and spatial distribution of gangliosides and ceramides in mouse model brains exposed to blast explosions. Ceramides were found to be present at higher concentrations in TBI brains; however, this was determined via ESI due to insufficient MALDI ionization efficiency. It was found that exposure to explosive blasts increases the amounts of a ganglioside GM2 in several portions of the brain and causes a subsequent decrease in the concentration of ceramide species (Figure 2). This was the first successful study to display an increase in GM2 from a non-genetic cause (Woods et al., 2013). The findings of Woods, et al. (2013) point towards the possibility of using gangliosides and ceramides as biomarkers in the detection and analysis traumatic brain injury in patients, and, furthermore, implicate MALDI-MSI as a useful strategy for biomarker discovery.

Medicinal Development

For the discovery and development of new drug entities, knowledge of a given compound's biodistribution is a critical factor. To where does a new drug compound distribute in the body? What are the metabolites of this new drug and where do these metabolites accumulate? What are the quantitative measures of their distribution? These questions must be answered in order to move from an *in vivo* animal model to human clinical trials. Because the approval process of a novel therapeutic is long, arduous, and very costly, areas in which technology can speed the acquisition of this information are open to modification (Castellino, 2012). The majority of these technology-driven areas throughout the drug development timeline occur within the pre-clinical stage. Traditionally, these early questions are answered via methods such as autoradiography and fluorescent tagging, each with its own set of limitations (Rasey et al., 1986, Solon et al., 2009). Both methods require labor-intensive synthesis efforts to apply the necessary tags. Autoradiography involves the use of radioactive materials, and thus comes with its own set of regulatory issues. When radiolabeled versions of novel compounds are delivered to an animal, an autoradiograph is produced; however, there is little to no indication of whether the observed image is the result of an intact compound or a metabolic fragment (Rasey et al., 1986, Solon et al., 2009).

Conversely, in a metabolite study, only metabolites containing radiolabeled atoms will be detected and observed. Fluorescence tags are significantly larger than radioactive labels, and require a significant amount of testing due to the possibility that the tag may interfere with the compound's membrane penetrance and efficacy (Rudin and Weissleder, 2003). Compounds are often less than 500 Da, meaning even the smallest dye molecule can have a large effect on the uptake and subsequent biodistribution of the compounds of interest. Images are further complicated by molecules exhibiting autofluorescence, and difficulties similar to radiolabeling, such as difficulty attributing a signal to intact or fragmented compound (Rudin and Weissleder, 2003). The synthesis of a pure compound itself provides a challenge.

These issues can be circumvented by MSI, as its non-targeted nature means all molecules on a given surface are chemically mapped, and no tag is required. With proper controls in place, previously unknown metabolites can be uncovered by utilizing tandem mass spectrometry (MS/MS) capabilities, essentially determining the structure of an unknown peak. Below we discuss a few case studies that have utilized MSI in drug development (Castellino, 2012, Castellino et al., 2011).

Fosdevirine

MALDI-MSI has already found success in the pharmaceutical industry, as evidenced by the recent study by Castellino, et al. (2013). Human immunodeficiency virus (HIV) is a widespread disease with limited treatments available. It is primarily an autoimmune disorder that develops when viral strains display varying resistance to the available treatments. It remains an active area of research in the pharmaceutical industry (Castellino et al., 2013).

The group of Castellino conducted experiments on a non-nucleoside reverse transcriptase inhibitor that had passed all pre-clinical testing and entered into Phase IIb clinical trials. Fosdevirine was developed by GlaxoSmithKline to treat a wide range of HIV-1 strains, the most common subtype as well as the most lethal. Many of these strains have become resistant to other forms of existing treatment, such as Efavirenz (Castellino et al., 2013). Rigorous pre-clinical testing identified Fosdevirine as a promising drug due to its efficacy in low doses, and its effectiveness against both single and double mutants of reverse transcriptase that confer remarkable resistance against other drugs. The drug moved into a human model, with Phase I testing conducted on healthy individuals indicating no observable toxicity or side effects. Further testing on HIV-1-infected individuals that had not previously received treatment confirmed earlier findings from healthy individuals. However, Phase IIb trials involving HIV-1-infected individuals that had undergone alternative treatments resulted in 25% of all subjects experiencing seizures, with no link to neurological problems in medical histories (Castellino et al., 2013).

MSI was employed to study its biodistribution and metabolism in rabbit, minipig, and monkey brain tissue in order to evaluate potential differences associated with adverse neurological symptoms. This was done in conjunction with liquid chromatography-MS (LC-MS) analyses of cerebral spinal fluid (CSF) from seizure patients as well as animal models. LC-MS analyses of CSF found two cysteine adduct metabolites, M22 and M16, to be present in seizure patients as well as rabbits and minipigs that exhibited central nervous

system (CNS) toxicity due to Fosveridine treatment. While these results indicate M22 and M16 as potential effectors of CNS toxicity, they do not provide spatial distribution information within the tissues themselves. MSI studies further indicated a potential mechanism for this CNS toxicity, providing important localization information. M22 was observed to localize to the white matter portion of the brain in minipig and rabbit, both of which exhibited Fosveridine-induced CNS toxicity. In monkeys, where no such toxicity was observed, Fosveridine was found to localize to the gray matter portion of the brain. It is hypothesized that binding of M22 and M16 to the GABA_A receptor could be the cause of this CNS toxicity. With MSI's capability to multiplex with other modes of imaging, it is possible to utilize a technique such as immunohistochemical staining in order to observe that M22 colocalizes with the GABA_A receptor in rabbit and minipig brain, but not monkey brain (Castellino et al., 2013). Together, this localization to the white matter and colocalization with the GABA_A receptor in model organisms exhibiting Fosveridine CNS toxicity show the advantages of combining MSI with traditional staining protocols (Castellino, 2012, Castellino et al., 2013, Castellino et al., 2011).

Paclitaxel

Optimized sample preparation protocols are important in the field of MSI. One such step is the matrix selection, both type and application method can be varied in order to optimize ionization of specific molecules (Reyzer and Caprioli, 2011, Rubakhin et al., 2005). This optimization is especially crucial in small drug molecule studies due to the ion suppression effects that are observed in the low *m/z* range of the spectrum. Because traditional MALDI matrices are, themselves, small molecules, they contribute a number of interfering, intense ion peaks in a mass spectrum. This can often suppress signal from target drug molecules that are present in much lower levels than the saturated matrix solutions. Additionally, the spatial resolution of any given imaging run can be limited by the size of the crystallized matrix particles themselves, often exceeding the size of the laser spot and thus decreasing the resolution of the data (Castellino, 2012, Castellino et al., 2011, Reyzer and Caprioli, 2011, Rubakhin et al., 2005).

One common solution to these problems is the use of nanoparticle-assisted laser desorption/ionization (NALDI). Nanoparticles are typically composed of an inorganic material, and exhibit such desirable characteristics as low heat capacity and efficient photo-adsorption. They are also smaller in size than matrix crystals, and thus do not interfere with spatial resolution as readily; additionally, they have a large surface area that allows for maximum adsorption of the desired analytes (Morosi et al., 2013).

Morosi, et al. (2013) employed NALDI for analysis of the biodistribution of a small drug molecule used for cancer treatments, Paclitaxel, in solid tumors. This group utilized MSI in organs excised from mice injected with Paclitaxel, and further utilized MSI in the study of human xenograft tumors excised from mice injected with Paclitaxel. MSI served two major advantages in the detection of Paclitaxel: negative ion mode and tandem mass spectrometry. Paclitaxel ionizes more efficiently in negative ion mode, and is typically suppressed by abundant lipids in positive ion mode over the *m/z* 800-1000 range (Paclitaxel MW 853.9 *m/z*). Thus, MSI allows for the option of collecting data for observation of a negatively

charged ion of Paclitaxel, specifically a fragment ion at m/z 284.2. MS/MS coupled to MSI experiments allowed for the confirmation of this peak by observing the transition from m/z 284.2 to m/z 72.6 (Morosi et al., 2013).

This group also employed quantitative MSI by utilizing a deuterated internal standard, D5-Paclitaxel. The internal standard was spotted onto control tissues, a calibration curve was constructed, and Paclitaxel signal was measured and compared to the internal standard signal in a specific region of interest. The success of this quantitative method was further shown by the differences in normalized intensities in liver when mice were treated with different levels of the drug. This group indicates that their success with quantitative MSI was likely due to the homogeneity of the nanoparticles used, versus the traditional heterogeneous MALDI matrix crystals (Morosi et al., 2013).

Read-Through Compounds

The treatment and management of genetic disorders are major target areas in the drug development field since no such drugs are yet of proven efficacy. One common genotype is caused by nonsense mutations, resulting in a malformed or unstable protein. Such nonsense mutations create a premature termination codon (PTC). Instead of translating a gene sequence in its entirety, the mRNA falls off the ribosome resulting in a truncated form of the protein. This protein fragment is subsequently degraded, and thus no full length, functional protein is produced by the patients' cells (Gatti, 2012).

Examples of PTC disorders include Duchenne muscular dystrophy (MD), cystic fibrosis, and ataxia-telangiectasia (A-T). A-T is often caused by a single point mutation in the ataxia-telangiectasia mutated (ATM) gene encoding for ATM protein. This serine/threonine kinase is active in DNA repair, mainly by phosphorylation of a large number of proteins involved in DNA repair and cancer. Individuals deficient in ATM exhibit such symptoms as impaired cerebellum development, increased risk of cancer (one-third of all A-T patients develop cancer), and enhanced susceptibility to radiation (Chun and Gatti, 2004, Chun et al., 2003, Swift et al., 1986).

Aminoglycosides, such as Gentamicin, could potentially be used to treat PTC disorders by binding to the small ribosomal subunit at the decoding site and inducing translational read-through of the stop codon (Yoshizawa et al., 1998). While this has been successful in laboratory studies in "proof of concept," it would engender adverse side effects such as toxicity and potential deafness (Barton-Davis et al., 1999, Lai et al., 2004, Zingman et al., 2007). Treating neurological diseases like A-T would also require that the aminoglycoside cross the blood-brain barrier. Due to their large and rigid structure, aminoglycosides do not penetrate a healthy blood-brain barrier. A new class of small molecule read-through (SMRT) compounds has recently been developed that overcomes most of these issues (Barton-Davis et al., 1999, Chun and Gatti, 2004, Chun et al., 2003, Du et al., 2009, Gatti, 2012, Lai et al., 2004, Swift et al., 1986, Yoshizawa et al., 1998, Zingman et al., 2007).

Although SMRT compounds have shown promise *in vitro*, there has been little information concerning the biodistribution of these compounds. In a mouse model, we determined the biodistribution of a derivative of RTC13 utilizing MSI (Du et al., 2009, Du et al., 2013). The

compound was administered intraperitoneally to mice, and its biodistribution was studied in all major organs at various time points. The SMRT compound was found to cross the blood-brain barrier without any off-target build-up. These results were confirmed by MS/MS, and tissue structure was elucidated by H&E staining, as shown in Figure 3 (in preparation). These results highlight the utility of MSI as an initial biodistribution screening technique due to its speed and direct analysis of unmodified, novel compounds.

Metals and Nanoparticles

A newer area of MSI being investigated is the imaging of nanoparticles and other metal elements. Metal analysis is typically done using inductively coupled plasma MS (ICP-MS), which uses a plasma flame to ionize metal particles wherein they are detected by a mass spectrometer (Zoriy et al., 2007, Zoriy et al., 2008). While ICP-MS is often coupled with liquid samples in an electrospray-like set-up, recent studies have utilized laser ablation ICP-MS (LA-ICP-MS) to study dried samples, such as droplets, tissues, and other surfaces. The coupling of ICP-MS to laser ablation has allowed for spatially relevant images to be collected, and thus MSI of metal elements is possible. We will discuss two examples of LA-ICP-MSI analyses of metals in tissue (Zoriy et al., 2007, Zoriy et al., 2008).

Aside from the study of innate metal composition of tissues, there is growing interest in metal- and non-metal-based nanoparticle biodistribution. This stems from the recent development of nanoparticles as drug delivery vehicles (Meng et al., 2010). Nanoparticles are promising due to their small, biocompatible nature and their ability to package many varieties of molecules, from proteins to small drug compounds, and deliver them to specific areas of a cell or organism. We will discuss a recent study that further stretched the capabilities of MALDI-MSI for the purposes of imaging nanoparticles in tissue (Yan et al., 2013).

Endogenous Metal Ions

LA-ICP-MS has long been used for the analysis of trace metals in samples; however, LA-ICP-MSI is a more recent application of this useful technique (Zoriy et al., 2007, Zoriy et al., 2008). Many metal ions play key roles in biological processes, thus it is of interest to study their distribution in tissue samples. Lear, et al. (2012) further improved the LA-ICP-MSI protocols in order to allow for the detection of traditionally undetectable metal ions by altering the reaction gas contents in a typical experiment.

Tissue surfaces are extremely complex, with thousands of different compounds present in a $1 \mu\text{m}^2$ pixel, thus it is advantageous to develop techniques that will allow for enhanced signal intensity of particular molecules of interest. Specifically in LA-ICP-MSI, argon gas is employed to provide electrons as well as assist in the nebulizing process of ions of interest; however, this use of Ar significantly interferes with a number of signal regions, most notably that of iron (Fe). Additional impurities in the Ar gas source can lead to further eclipsing of signals of interest. This group employed the use of an H_2 reaction gas to dissociate ArO clusters and other interfering compounds while maintaining sensitivity in the detection of several metal ions, including: Mn, Fe, Cu, and Zn (Lear et al., 2012).

Mouse brain was used to study the distribution of each of the above mentioned metal ions. For Cu and Zn ions, specifically ^{63}Cu and ^{66}Zn , there are no known interferences and thus it was shown that use of H_2 reaction gas did not improve signal intensity during an LA-ICP-MSI experiment; however, both ions were able to be mapped at a 30 μm spatial resolution. The success of the H_2 reaction gas was evident in the analysis of the ^{56}Fe and ^{57}Fe ions, as well as the ^{55}Mn ion, both of which experience heavy interference with Ar gas clusters and impurities. Background signal was reduced after introduction of the reaction gas, and thus a much clearer ^{56}Fe image was produced at a spatial resolution of 6 μm . This improvement was even more pronounced in the analysis of ^{57}Fe , which is more heavily suppressed by interfering signals due to its lower abundance. The adaptability of this technique for analysis of important metal ions indicates its promise as an analytical tool for analysis of all biologically relevant molecules, from metal ions to proteins (Lear et al., 2012).

Platinum from cis-platin drug

Drug toxicity studies are imperative in advancing novel compounds into clinical trials. *Cis*-platin is a commonly used drug complex in the treatment of many types of cancers; however, it is known to exhibit nephrotoxicity when given in larger doses. It is of great interest to observe the biodistribution of platinum (Pt) in *cis*-platin-treated individuals, as well as the differential distributions of copper and zinc (Cu and Zn), two important metal ions involved in biological processes (Zoriy et al., 2007).

Traditionally, Pt has been tracked in tissue via neutron activation analysis and autoradiography. These techniques, while sensitive, require labor-intensive techniques further complicated by the need for a nuclear reactor to produce neutrons. Less expensive alternatives to nuclear reactors often result in lower sensitivity, and still involve the use of radioactive materials. Zoriy, et al. (2007) turned to LA-ICP-MSI to study the biodistribution of Pt, a technique that does not require the use of radioactive materials and is capable of whole-tissue analysis. This technique had been previously employed to show a correlation between the Alzheimer's disease-related amyloid beta protein ($\text{A}\beta$) and trace element concentration (Hutchinson et al., 2005).

This group was able to image the biodistribution of Pt, Cu, and Zn in mouse kidney derived from *cis*-platin-dosed animals. Hematoxylin and eosin staining was used to distinguish specific kidney structures, with LA-ICP-MSI experiments utilizing a spatial resolution of 50 μm . Zoriy, et al. (2007) were also able to employ internal standards for quantitative purposes. They observed a higher concentration of Cu in the capsule and external cortex (glomeruli), and a higher concentration of Zn in the inner cortex (tubules). Pt concentration was highest in the medulla, decreased in the inner cortex, and was lowest along the periphery of the kidney. The Pt gradient reflected the typical primary urine gradient found in the kidney structures. This is thought to be the first alternative imaging method for Pt that did not require the use of radioactive materials, and thus shows the versatility of MSI (Zoriy et al., 2007).

Nanoparticles

A growing problem in medicine is the treatment of drug-resistant diseases, especially drug-resistant cancers. These cancers often require a combination of drugs working synergistically to effectively kill the fast-growing cells. Determining the perfect treatment cocktail can be a daunting task for physicians and researchers, and further prescribing of a large number of medications can create a variety of burdens for the patient in question. This also leads to complications in FDA investigational new drug (IND) studies. Nanoparticle drug delivery vehicles are a promising solution to these woes for a number of reasons (Meng et al., 2010).

Nanoparticles are typically small, biocompatible molecules on the scale of nanometers. Their surfaces are easily modified via various synthesis routes, and this allows for specific targeting of nanoparticles to desired cell types (Meng et al., 2010). Additionally, their mostly hollow cores allow for packaging of a variety of molecules. Recent studies have indicated nanoparticles as effective delivery vehicles to carry molecules to specific drug-resistant cancer cells and subsequently release them, in this particular case these molecules included siRNA and a small drug compound (Meng et al., 2010).

Traditional nanoparticle imaging methods require the use of a large dye molecule, which can interfere with the activity and resulting biodistribution of the nanoparticles in question. Nanoparticles, such as gold or silica, are often used as MALDI matrices due to their limited background noise contributions in a mass spectrum (Morosi et al., 2013). However, they are not commonly detected using LDI-based methods due to their variation in size, the fact that they often contain fixed charges, and the lack of a predictive spectral profile.

Yan, et al. (2013) have circumvented these limitations by establishing a spectral profile, or 'barcode,' of the nanoparticles involved via attachment of a ligand molecule to the surface of the nanoparticle. These ligands are smaller in size than traditional dyes, and serve the added function of modifying the surface properties of the nanoparticles. This study was able to identify a molecular ion attributed to the gold nanoparticle core, as well as three distinct molecular ions attributed to each of the ligands attached, as shown in Figure 4. This illustrates a promising future for the use of LDI-MS-based imaging methods for the detection of such nontraditional molecules as nanoparticles (Yan et al., 2013).

Conclusion

Since its inception nearly two decades ago, the field of mass spectrometry imaging has grown rapidly. New developments in sample preparation protocols, desorption/ionization methods, instrumentation (e.g., mass spectrometry analyzers and detectors), and data processing and data display have aided in its incorporation into bioanalytical chemistry and even medicine. We have discussed a number of applications in the realm of small molecules, and many more examples of MSI's utility can be found in the study of peptides and proteins. Going forward, it is likely that MSI will become a permanent fixture in biomedical research. Further advancements in software and instrumentation are already aiding its incorporation into clinical settings, and the continued, collaborative goal towards standardized protocols will aid in its development as a pharmaceutical and clinical tool.

Acknowledgments

This work was supported by the Ruth L. Kirschstein National Research Service Award (Grant GM007185, UCLA Cellular and Molecular Biology Training Grant, for C.N.F.) and the US National Institutes of Health Shared Instrumentation Program (Grant S10 RR025600 to J.A.L.).

References

- ANDERSSON M, GROSECLOSE MR, DEUTCH AY, CAPRIOLI RM. Imaging mass spectrometry of proteins and peptides: 3D volume reconstruction. *Nat Meth.* 2008; 5:101–108.
- BARTON-DAVIS ER, SHOTURMA DI, SWEENEY HL. Contribution of satellite cells to IGF-I induced hypertrophy of skeletal muscle. *Acta Physiol Scand.* 1999; 167:301–5. [PubMed: 10632630]
- BÖRNER K, MALMBERG P, MÅNSSON J-E, NYGREN H. Molecular imaging of lipids in cells and tissues. *International Journal of Mass Spectrometry.* 2007; 260:128–136.
- BUCCOLIERO R, FUTERMAN AH. The roles of ceramide and complex sphingolipids in neuronal cell function. *Pharmacological Research.* 2003; 47:409–419. [PubMed: 12676515]
- BULLEY NR, FATTORI M, MEISEN A, MOYLS L. Supercritical fluid extraction of vegetable oil seeds. *Journal of the American Oil Chemists' Society.* 1984; 61:1362–1365.
- CASTELLINO S. MALDI imaging MS analysis of drug distribution in tissue: the right time!(!?). *Bioanalysis.* 2012; 4:2549–2551. [PubMed: 23173789]
- CASTELLINO S, GROSECLOSE MR, SIGAFOOS J, WAGNER D, DE SERRES M, POLLI JW, ROMACH E, MYER J, HAMILTON B. Central Nervous System Disposition and Metabolism of Fosdevirine (GSK2248761), a Non-Nucleoside Reverse Transcriptase Inhibitor: An LC-MS and Matrix-Assisted Laser Desorption/Ionization Imaging MS Investigation into Central Nervous System Toxicity. *Chemical Research in Toxicology.* 2013; 26:241–251. [PubMed: 23227887]
- CASTELLINO S, GROSECLOSE MR, WAGNER D. MALDI imaging mass spectrometry: bridging biology and chemistry in drug development. *Bioanalysis.* 2011; 3:2427–41. [PubMed: 22074284]
- CHAUHAND P, CORNETT DS, CAPRIOLI RM. Molecular imaging of thin mammalian tissue sections by mass spectrometry. *Current Opinion in Biotechnology.* 2006; 17:431–436. [PubMed: 16781865]
- CHUN HH, GATTI RA. Ataxia-telangiectasia, an evolving phenotype. *DNA Repair (Amst).* 2004; 3:1187–96. [PubMed: 15279807]
- CHUN HH, SUN X, NAHAS SA, TERAOKA S, LAI C-H, CONCANNON P, GATTI RA. Improved diagnostic testing for ataxia-telangiectasia by immunoblotting of nuclear lysates for ATM protein expression. *Molecular Genetics and Metabolism.* 2003; 80:437–443. [PubMed: 14654357]
- COLSCH B, JACKSON SN, DUTTA S, WOODS AS. Molecular Microscopy of Brain Gangliosides: Illustrating their Distribution in Hippocampal Cell Layers. *ACS Chemical Neuroscience.* 2011; 2:213–222. [PubMed: 21961052]
- CONTE E, MILANI R, MORALI G, ABBALLE F. Comparison between accelerated solvent extraction and traditional extraction methods for the analysis of the herbicide diflufenican in soil. *Journal of Chromatography A.* 1997; 765:121–125.
- COONS AH, CREECH HJ, JONES RN, BERLINER E. The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody. *J. Immunol.* 1942; 45:159–70.
- DU L, DAMOISEAUX R, NAHAS S, GAO K, HU H, POLLARD JM, GOLDSTINE J, JUNG ME, HENNING SM, BERTONI C, GATTI RA. Nonaminoglycoside compounds induce readthrough of nonsense mutations. *J Exp Med.* 2009; 206:2285–97. [PubMed: 19770270]
- DU L, JUNG ME, DAMOISEAUX R, COMPLETO G, FIKE F, KU JM, NAHAS S, PIAO C, HU H, GATTI RA. A New Series of Small Molecular Weight Compounds Induce Read Through of All Three Types of Nonsense Mutations in the ATM Gene. *Mol Ther.* 2013
- EBERLIN LS, FERREIRA CR, DILL AL, IFA DR, CHENG L, COOKS RG. Nondestructive, Histologically Compatible Tissue Imaging by Desorption Electrospray Ionization Mass Spectrometry. *ChemBioChem.* 2011a; 12:2129–2132. [PubMed: 21793152]

- EBERLIN LS, LIU X, FERREIRA CR, SANTAGATA S, AGAR NYR, COOKS RG. Desorption Electrospray Ionization then MALDI Mass Spectrometry Imaging of Lipid and Protein Distributions in Single Tissue Sections. *Analytical Chemistry*. 2011b; 83:8366–8371. [PubMed: 21975048]
- EBERLIN LS, NORTON I, ORRINGER D, DUNN IF, LIU X, IDE JL, JARMUSCH AK, LIGON KL, JOLESZ FA, GOLBY AJ, SANTAGATA S, AGAR NYR, COOKS RG. Ambient mass spectrometry for the intraoperative molecular diagnosis of human brain tumors. *Proceedings of the National Academy of Sciences*. 2013; 110:1611–1616.
- ELLIS SR, BROWN SH, IN HET PANHUIS M, BLANKSBY SJ, MITCHELL TW. Surface analysis of lipids by mass spectrometry: More than just imaging. *Progress in Lipid Research*. 2013; 52:329–353. [PubMed: 23623802]
- FAHY E, SUBRAMANIAM S, BROWN HA, GLASS CK, MERRILL AH, MURPHY RC, RAETZ CRH, RUSSELL DW, SEYAMA Y, SHAW W, SHIMIZU T, SPENER F, VAN MEER G, VANNIEUWENHZE MS, WHITE SH, WITZTUM JL, DENNIS EA. A comprehensive classification system for lipids. *Journal of Lipid Research*. 2005; 46:839–862. [PubMed: 15722563]
- FERGUSON L, BRADSHAW R, WOLSTENHOLME R, CLENCH M, FRANCESE S. Two-Step Matrix Application for the Enhancement and Imaging of Latent Fingerprints. *Analytical Chemistry*. 2011; 83:5585–5591. [PubMed: 21667965]
- FUCHS B, SÜS R, SCHILLER J. An update of MALDI-TOF mass spectrometry in lipid research. *Progress in Lipid Research*. 2010; 49:450–475. [PubMed: 20643161]
- GARCÍA-AYUSO LE, VELASCO J, DOBARGANES MC, LUQUE DE CASTRO MD. Determination of the oil content of seeds by focused microwave-assisted soxhlet extraction. *Chromatographia*. 2000; 52:103–108.
- GATTI RA. SMRT compounds correct nonsense mutations in primary immunodeficiency and other genetic models. *Ann N Y Acad Sci*. 2012; 1250:33–40. [PubMed: 22364446]
- HOLTHUIS JCM, POMORSKI T, RAGGERS RJ, SPRONG H, VAN MEER G. The Organizing Potential of Sphingolipids in Intracellular Membrane Transport. *Physiological Reviews*. 2001; 81:1689–1723. [PubMed: 11581500]
- HUTCHINSON RW, COX AG, MCLEOD CW, MARSHALL PS, HARPER A, DAWSON EL, HOWLETT DR. Imaging and spatial distribution of β -amyloid peptide and metal ions in Alzheimer's plaques by laser ablation–inductively coupled plasma–mass spectrometry. *Analytical Biochemistry*. 2005; 346:225–233. [PubMed: 16214103]
- LAI CH, CHUN HH, NAHAS SA, MITUI M, GAMO KM, DU L, GATTI RA. Correction of ATM gene function by aminoglycoside-induced read-through of premature termination codons. *Proc Natl Acad Sci U S A*. 2004; 101:15676–81. [PubMed: 15498871]
- LAZOVA R, SEELEY EH, KEENAN M, GUEORGUEVA R, CAPRIOLI RM. Imaging Mass Spectrometry—A New and Promising Method to Differentiate Spitz Nevi From Spitzoid Malignant Melanomas. *The American Journal of Dermatopathology*. 2012; 34:82–90. [PubMed: 22197864]
- LEAR J, HARE DJ, FRYER F, ADLARD PA, FINKELSTEIN DI, DOBLE PA. High-Resolution Elemental Bioimaging of Ca, Mn, Fe, Co, Cu, and Zn Employing LA-ICP-MS and Hydrogen Reaction Gas. *Analytical Chemistry*. 2012; 84:6707–6714. [PubMed: 22746971]
- MARSHALL AG, HENDRICKSON CL, JACKSON GS. Fourier transform ion cyclotron resonance mass spectrometry: A primer. *Mass Spectrometry Reviews*. 1998; 17:1–35. [PubMed: 9768511]
- MCCOMBIE G, KNOCHENMUSS R. Small-Molecule MALDI Using the Matrix Suppression Effect To Reduce or Eliminate Matrix Background Interferences. *Analytical Chemistry*. 2004; 76:4990–4997. [PubMed: 15373433]
- MCDONNELL LA, HEEREN RM. Imaging mass spectrometry. *Mass Spectrom Rev*. 2007a; 26:606–43. [PubMed: 17471576]
- MCDONNELL LA, HEEREN RMA. Imaging mass spectrometry. *Mass Spectrometry Reviews*. 2007b; 26:606–643. [PubMed: 17471576]

- MENG H, LIONG M, XIA T, LI Z, JI Z, ZINK JI, NEL AE. Engineered design of mesoporous silica nanoparticles to deliver doxorubicin and P-glycoprotein siRNA to overcome drug resistance in a cancer cell line. *ACS Nano*. 2010; 4:4539–50. [PubMed: 20731437]
- MOROSI L, SPINELLI P, ZUCCHETTI M, PRETTO F, CARRÀ A, D'INCALCI M, GIAVAZZI R, DAVOLI E. Determination of Paclitaxel Distribution in Solid Tumors by Nano-Particle Assisted Laser Desorption Ionization Mass Spectrometry Imaging. *PLoS ONE*. 2013; 8:e72532. [PubMed: 23991120]
- MURPHY RC, HANKIN JA, BARKLEY RM. Imaging of lipid species by MALDI mass spectrometry. *Journal of Lipid Research*. 2009; 50:S317–S322. [PubMed: 19050313]
- RASEY JS, KROHN KA, GRUNBAUM Z, SPENCE AM, MENARD TW, WADE RA. Synthesis, biodistribution, and autoradiography of radiolabeled S-2-(3-methylaminopropylamino)ethylphosphorothioic acid (WR-3689). *Radiat Res*. 1986; 106:366–79. [PubMed: 3012625]
- REYZER, M.; CAPRIOLI, R. Imaging Mass Spectrometry.. In: BANOUB, J., editor. *Detection of Biological Agents for the Prevention of Bioterrorism*. Springer; Netherlands: 2011.
- RUBAKHIN SS, JURCHEN JC, MONROE EB, SWEEDLER JV. Imaging mass spectrometry: fundamentals and applications to drug discovery. *Drug Discovery Today*. 2005; 10:823–837. [PubMed: 15970265]
- RUDIN M, WEISSLEDER R. Molecular imaging in drug discovery and development. *Nat Rev Drug Discov*. 2003; 2:123–131. [PubMed: 12563303]
- RUJOI M, ESTRADA R, YAPPERT MC. In Situ MALDI-TOF MS Regional Analysis of Neutral Phospholipids in Lens Tissue. *Analytical Chemistry*. 2004; 76:1657–1663. [PubMed: 15018564]
- SANCHEZ-CARBAYO M. Antibody Arrays: Technical Considerations and Clinical Applications in Cancer. *Clinical Chemistry*. 2006; 52:1651–1659. [PubMed: 16809399]
- SCHULTZ C, NEEF AB, GADELLA TW, GOEDHART J. Imaging Lipids in Living Cells. *Cold Spring Harbor Protocols*. 2010; 2010.pdb.top83.
- SCHWAMBORN K, CAPRIOLI RM. MALDI Imaging Mass Spectrometry – Painting Molecular Pictures. *Molecular Oncology*. 2010; 4:529–538. [PubMed: 20965799]
- SOLON EG, SCHWEITZER A, STOECKLI M, PRIDEAUX B. Autoradiography, MALDI-MS, and SIMS-MS Imaging in Pharmaceutical Discovery and Development. *The AAPS Journal*. 2009; 12:11–26. [PubMed: 19921438]
- SWIFT M, MORRELL D, CROMARTIE E, CHAMBERLIN AR, SKOLNICK MH, BISHOP DT. The incidence and gene frequency of ataxiatelangiectasia in the United States. *Am J Hum Genet*. 1986; 39:573–83. [PubMed: 3788973]
- TAYLOR S, KING J, LIST G. Determination of oil content in oilseeds by analytical supercritical fluid extraction. *Journal of the American Oil Chemists' Society*. 1993; 70:437–439.
- WANG H-YJ, LIU CB, WU H-W. A simple desalting method for direct MALDI mass spectrometry profiling of tissue lipids. *Journal of Lipid Research*. 2011; 52:840–849. [PubMed: 21266365]
- WATROUS JD, ALEXANDROV T, DORRESTEIN PC. The evolving field of imaging mass spectrometry and its impact on future biological research. *Journal of Mass Spectrometry*. 2011; 46:209–222. [PubMed: 21322093]
- WENK MR. The emerging field of lipidomics. *Nat Rev Drug Discov*. 2005; 4:594–610. [PubMed: 16052242]
- WOODS AS, COLSCH B, JACKSON SN, POST J, BALDWIN K, ROUX A, HOFFER B, COX BM, HOFFER M, RUBOVITCH V, PICK CG, SCHULTZ JA, BALABAN C. Gangliosides and Ceramides Change in a Mouse Model of Blast Induced Traumatic Brain Injury. *ACS Chemical Neuroscience*. 2013; 4:594–600. [PubMed: 23590251]
- YAN B, KIM ST, KIM CS, SAHA K, MOYANO DF, XING Y, JIANG Y, ROBERTS AL, ALFONSO FS, ROTELLO VM, VACHET RW. Multiplexed Imaging of Nanoparticles in Tissues Using Laser Desorption/Ionization Mass Spectrometry. *Journal of the American Chemical Society*. 2013; 135:12564–12567. [PubMed: 23931011]
- YOSHIZAWA S, FOURMY D, PUGLISI JD. Structural origins of gentamicin antibiotic action. *EMBO J*. 1998; 17:6437–48. [PubMed: 9822590]

- ZEMSKI BERRY KA, HANKIN JA, BARKLEY RM, SPRAGGINS JM, CAPRIOLI RM, MURPHY RC. MALDI Imaging of Lipid Biochemistry in Tissues by Mass Spectrometry. *Chemical Reviews*. 2011; 111:6491–6512. [PubMed: 21942646]
- ZINGMAN LV, PARK S, OLSON TM, ALEKSEEV AE, TERZIC A. Aminoglycoside-induced translational read-through in disease: overcoming nonsense mutations by pharmacogenetic therapy. *Clin Pharmacol Ther*. 2007; 81:99–103. [PubMed: 17186006]
- ZORIY M, MATUSCH A, SPRUSS T, BECKER JS. Laser ablation inductively coupled plasma mass spectrometry for imaging of copper, zinc, and platinum in thin sections of a kidney from a mouse treated with cisplatin. *International Journal of Mass Spectrometry*. 2007; 260:102–106.
- ZORIY MV, DEHNHARDT M, MATUSCH A, BECKER JS. Comparative imaging of P, S, Fe, Cu, Zn and C in thin sections of rat brain tumor as well as control tissues by laser ablation inductively coupled plasma mass spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy*. 2008; 63:375–382.

Figure 1.

Schematic of a MALDI-MSI experiment shows a typical tissue sample preparation protocol. A laser then rasters across the tissue surface. At each point, or pixel, a mass spectrum is collected. These spectra are averaged, and individual masses are selected to create maps, such as the one shown on the right (kidney tissue of a small molecule metabolite).

Figure 2.

MALDI-MSI of two gangliosides: (a) GD1d18:1/C18:0 and (b) GM2 d18:1/C18:0 in brains of control mice and mice 2, 24, and 72 h after open field blast exposure at 4 and 7 m. In panel a, GD1 is present mainly in gray matter areas (hippocampus, cortex, and hypothalamus), and there is no observable change in the distribution of GD1 between control and blast groups. In panel b, for the controls, the GM2 peak was highly localized in the lateral and the dorsal third ventricles while increases were observed in the hippocampus and thalamus for blast exposure, especially for 4 m 2 and 24 h postblast samples. Reprinted

with permission from the *Journal of the American Chemical Society*. Copyright © 2013 American Chemical Society (Woods et al., 2013).

Figure 3.

MALDI-MSI of brains from SMRT-treated mice. The top panel shows a sodiated adduct found in cerebellum, imaging shown on right: green spots (white arrows) correspond to signal. Tissue was H&E stained following MSI. The bottom panel shows a sodiated adduct found in the cerebrum.

Figure 4.

Structures of the surface monolayers on the AuNPs: the “mass barcode” is the m/z of the AuNP surface ligand. LDI-MS images of AuNPs in mouse spleens. The biodistributions of AuNPs are shown in panel (a) (AuNP 1), (b) (AuNP 2), and (c) (AuNP 3). Reprinted and adapted with permission from the *Journal of the American Chemical Society*. Copyright © 2013 American Chemical Society (Yan et al., 2013).